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Seroprevalence of Bovine Leukemia Virus in Some Dairy Farms in Iran

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Abstract: A seroepidemiological survey of bovine leukemia virus (BLV) infection was conducted in some dairy farms during July 2010 to January 2011, using an enzyme-linked immunosorbent assay (ELISA) test. A total of 137 cattle were tested. The overall prevalence of BLV infection was 29.9%. The prevalence of BLV infection in general dairy farms (43.9%) was statistically higher than private dairy farms (0.04%)($P< 0.001$). Analysis of the relationships between age and parity to Enzootic bovine leukosis (EBL) in sero-positive herds indicated significant associations ($P< 0.05$). Further investigation is required to determine the risk factors necessary to control BLV infection that may take into account the different farming practices that exist between dairy farms.

Key words: Bovine Leukemia Virus · EBL · Seroprevalence · Dairy Cattle · Iran

INTRODUCTION

Enzootic bovine leukosis (EBL), the most frequent bovine neoplastic disease, has a worldwide distribution [1]. Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis, a neoplasm of lymphatic tissue in bovine species in the genus *Deltaretrovirus*, *Retroviridae*. It is closely related to human T cell leukemia virus types 1 and 2 (HTLV-1 and 2) [1, 2]. Cattle infected with BLV usually show no clinical sign; only 30-70% of infected animals develop persistent lymphocytosis and 0.1-10% develop malignant lymphosarcoma [3, 4]. The disease may also follow an asymptomatic course, in which the infected animals act as carriers without showing any symptoms of disease. In addition to losses caused by death due to lymphosarcoma, live cattle as well as their semen and ova from seropositive cattle, are ineligible for export to many countries [5].

BLV-exposed cattle become life-long virus carriers [6]. The virus is present in blood lymphocytes and in tumor cells as provirus integrated into the genome and found in the cellular fraction of various body fluids. Since transmission of BLV may occur via contact with the affected animals; parturition, mechanical transmission by insects, blood transfusion and the use of common needles, it is emphasized to carry out surveillance study for the control of the disease [7]. Disease transmission between cattle is considered to occur via exposure to infected lymphocytes [1, 5]. Bovine leukemia virus may be transmitted both vertically and horizontally; however, horizontal transmission is considered to be more important, usually occurring through poor management and manipulation [8]. Although most BLV infected cattle do not develop apparent clinical signs, BLV is considered to potentially induce significant economical losses due to the reduction in milk production, reproductive performance and period of life [9, 10].

It is well known that, like other slow virus infections, EBL infection also spreads slowly over long periods. Once the cattle population is infected by this virus, the achievement of eradication is thought to be difficult. Because no vaccine is available, virus specific antibodies found in serum or milk a good indicator of exposure and a practical method for disease screening [11]. Studies from other regions of Iran on seroprevalence of EBL have reported cattle prevalence rates of 3% in Central province [12], 5.7% in Charmahal-o-Bakhtiari province [13] and 0.5% in city of Ahwaz [14]. EBL was successfully eradicated through national control programs in some European countries in recent years [15].

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The aim of this study was to investigate the seroprevalence of BLV infection in dairy cattle from private and general dairy farms in Iran, using ELISA test.

MATERIALS AND METHODS

Blood Samples Collection: The present study was performed on Holstein dairy herds. Blood samples were obtained from farms on a voluntary basis from July 2010 to January 2011. Cattle over 2 years old were sampled in the selected farms. Cattle were randomly selected. Under these criteria, a total of 137 cattle (89 from general farm and 48 from private farm) were subjected to testing.

Blood was aseptically taken from jugular vein with and without anticoagulant. Blood samples without anticoagulant were allowed to clot at room temperature and centrifuged at 1500g for 15 min for serum collection. Serum samples were separated and stored at-20°C until analysis.

ELISA Test: ELISA was performed on commercially available micro plates for the detection of the anti-BLV gp51 antibody according to the manufacturer's instructions (Institute Pourquier, Montpellier, France). The sensitivity and specificity of the ELISA test were 99.0 and 99.6%, respectively [16]. Briefly, 50 µl of wash solution (PBS-T: 10 mM phosphate buffer pH 7.4, 150 mM NaCl, 0.05% Tween 20) was added in each well and 50 µl of the test sera. Positive and negative control sera were also added in appropriate wells. The plates were washed with wash solution three times (solution supplied by kit) after incubation at 25 for 30 minutes. One hundred µl of anti-gp51 horseradish peroxidase (HRP) conjugate was added into wells at dilution of 1:100 and incubated at room temperature for 1 hour. The plates were then washed three times with washing solution. Two hundreds µl tetra methyl benzidine (TMB) substrate was added in each well and incubated for 20 minutes at room temperature, away from direct light to realize the reaction. Finally, 100 µl of stop solution was dispensed per well to stop the reaction. The reaction was read by an ELISA reader (Stat Fax 2100, Awareness Technology Inc, USA) at 405 optical density (OD). The net extinction value was calculated according to the formula supplied by the manufacturer.

Hematological Tests: Erythrocyte, total leukocyte, packed cell volume and hemoglobin in blood samples with anticoagulant were immediately measured using an automatic analyzer (Abacus, Hema Screen; Hospitex Diagnostics, Italy). Persistent Lymphocytosis (PL) was defined by a property of lymphocyte counts of more than $10^4\ \mu l^{-1}$ in peripheral blood [17].

Statistical Analysis: Statistical analyses were carried out using SPSS version 16 (SPSS Inc, Chicago, Illinois, USA). The chi-square test and analysis of variance were used to compare the results of categorical variables. Logistic regression analysis was used to evaluate the effects of age and parity on infection. Statistical significance was set at $P< 0.05$.

RESULTS

One way ANOVA revealed no significant differences among four groups regarding age and parity.

Forty one out of 137 (29.9%) sampled cows were seropositive to BLV. Most seropositive animals were found in the general farms. In these farms, prevalence of BLV positive cattle was 42.6% and 45.2% (Table 1). Logistic analysis regression showed that age and parity were significant variables in seropositivity ($P= 0.002$), so that, with increase of age risk of BLV-positivity was increased.

There were statistically significant differences in percentage of infection between groups ($P< 0.05$) (Table 1). The highest percentage of infection among cattle of farms 1 and 2 (general farms) with frequency of 45.2% and 42.6% were seen but farms 3 and 4 (Private farms) had significantly lower percentage of infection (8% and 0%) ($P< 0.001$). The PL difference between these two kinds of farms was not statistically significant ($P= 0.118$).

Table 1: Seroprevalence of BLV among dairy cattle farms

Type of farms	Farm Number	Age Means ±SD	Parity Means ±SD	No. (%) of BLV-Positive	No. (%) Of PL-Positive
Governmental	1	3.22^{a} ±1.59	3.22^{a} ±1.58	42* (45.2 %)	42^{ns} (11.9 %)
farms	2	$2.79^{a,c}$ ±1.97	$2.79^{a,b}$ ±1.96	47* (42.6 %)	47^{ns} (10.6 %)
Private	3	3.88^{a} ±1.90	3.88^{a} ±1.90	25** (8 %)	25^{ns} (0 %)
farms	4	2.09^{c} ±0.90	2.09^{b} ±0.90	23** (0 %)	23^{ns} (0 %)

Mean values with the same small letter superscript are not significantly different ($P > 0.05$)

*= $P < 0.05$, ** = $P < 0.01$, ns = $P > 0.05$.

PL: Persistent Lymphocytosis

DISCUSSION

In the present study, 29.9% BLV seropositivity was detected among the sampled cattle population. Previous study on abattoir prevalence of bovine leukemia virus in Holstein cattle of these farms has reported cattle seroprevalence rates of 22.3% in 197 cattle [18]. This high prevalence of infected dairy cattle was also observed in previous report in the USA and Japan [19, 20]. Previous studies on seroprevalence of EBL in different localities in Turkey (neighbor of Iran) have reported cattle prevalence rates of 33% in Karacabey, 30% in Cukurova [21], 18% in eastern Turkey [22], 10.6% in Trakya district, Marmara region [23] and 9% in Bursa [24]. On the other hand, Low prevalence was observed in some countries of the European Union, where EBL cattle prevalence are rarely found to be < 1.5% [25].

A further finding of this study was the presence of an association between age and EBL seropositivity in infected herds which has been shown in other studies. Besides, parity was also another significant variable which indirectly implied aging. EBL occurs rarely in animals less than 2 years of age and the incidence increases with increasing age [25].

We observed that the prevalence of BLV infection was more in general farms. Interestingly, there was a significant difference in the prevalence of BLV between general and private dairy farms. The reason may be derived from the different management practices of dairy farms. There are an important difference in term of facilitating the management and the sanitary control of both types of farms. In other words, general farms have poor condition of sanitation and high density population. It appears that close physical contact and exchange of contaminated biological materials are required for transmission. The virus is present mostly in lymphocytes and can be found in the blood, milk and tumor masses. Most susceptible cattle become infected by exposure to infected lymphocytes and not by cell-free virus. Therefore, any concept by which BLV-infected lymphocytes can be transmitted from one cow to another is a potential meaning of transmission [26]. This study has shown that EBL is prevalent in Iran. The costs and benefits from eradicating the disease in this region should be carefully evaluated. In the absence of an effective vaccine, eradication strategies used in other countries have been based on a policy of sero grouping followed by segregating or culling seropositive animals and implementing corrective action including sero grouping and isolating new infected animal from the herd and applying strict measures to prevent iatrogenic transmission [25, 27]. An eradication plan in Iran, based on “sero grouping and segregation” would face the added difficulty of separating seropositive from seronegative cattle from different herds and therefore, would require a conscientious and well-coordinated effort from all cattle owners in the district. The alternative policy of sero grouping and culling seropositive animals has been successful at eradicating EBL in some European countries with a low prevalence of animals with persistent lymphocytosis and tumorous leukosis [25]. Based on other study, examination of bull's semen samples that used for artificial insemination for the control and prevention of bovine leukosis infection in cattle seems to be necessary [28].

Although the reason for the spread of BLV infection was not clarified in this study, further investigations considering different farming practices of the dairy farms are likely to elucidate the key risk factors of BLV transmission in an attempt to control new infections. Furthermore, it will be essential to conduct nationwide surveillance to more accurately estimate BLV prevalence and the major risk factors.

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